Environmental Research 148 (2016) 256–263

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Short-term health effects in the general population following a major train accident with acrylonitrile in Belgium

K. Simons [a,1,2], T. De Smedt [a,1,2], C. Stove [b,3], P. De Paepe [c,4], M. Bader [d,5], B. Nemery [e,6], C. Vleminckx [a,2], K. De Cremer [a,2], I. Van Overmeire [a,2], S. Fierens [a,2], B. Mertens [a,2], T. Göen [f,7], T. Schettgen [g,8], H. Van Oyen [a,2], J. Van Loco [a,2], A. Van Nieuwenhuyse [a,*,2]

[a] Scientific Institute of Public Health, Brussels, Belgium
[b] Ghent University, Laboratory of Toxicology, Department of Bioanalysis, Ghent, Belgium
[c] Ghent University Hospital, Department of Emergency Medicine, Ghent, Belgium
[d] BASF SE, Occupational Medicine & Health Protection, Ludwigshafen, Germany
[e] Katholieke Universiteit Leuven, Department of Public Health and Primary Care, Centre for Environment and Health, Leuven, Belgium
[f] University of Erlangen-Nuremberg, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Erlangen, Germany
[g] RWTH Aachen University, Institute for Occupational and Social Medicine, Medical Faculty, Aachen, Germany

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
22 March 2016
Accepted 23 March 2016
Available online 15 April 2016

Keywords:
Acrylonitrile
Human biomonitoring
Health effects
Sewage
General population
Accident

ABSTRACT

Background: Following a train derailment, several tons of acrylonitrile (ACN) exploded, inflamed and part of the ACN ended up in the sewage system of the village of Wetteren. More than 2000 residents living in the close vicinity of the accident and along the sewage system were evacuated. A human biomonitoring study of the adduct *N*-2-cyanoethylvaline (CEV) was carried out days 14–21 after the accident.
Objectives: (1) To describe the short-term health effects that were reported by the evacuated residents following the train accident, and (2) to explore the association between the CEV concentrations, extrapolated at the time of the accident, and the self-reported short-term health effects.
Methods: Short-term health effects were reported in a questionnaire (n=191). An omnibus test of independence was used to investigate the association between the CEV concentrations and the symptoms. Dose-response relationships were quantified by Generalized Additive Models (GAMs).
Results: The most frequently reported symptoms were local symptoms of irritation. In non-smokers, dose-dependency was observed between the CEV levels and the self-reporting of irritation (p=0.007) and nausea (p=0.007). Almost all non-smokers with CEV concentrations above 100 pmol/g globin reported irritation symptoms. Both absence and presence of symptoms was reported by non-smokers with CEV concentrations below the reference value and up to 10 times the reference value. Residents who visited the emergency services reported more symptoms. This trend was seen for the whole range of CEV concentrations, and thus independently of the dose.
Discussion and conclusion: The present study is one of the first to relate exposure levels to a chemical released during a chemical incident to short-term (self-reported) health effects. A dose-response relation was observed between the CEV concentrations and the reporting of short-term health effects in the non-

* Correspondence to: Scientific Institute of Public Health, J. Wytsmanstraat 14, 1050 Brussels, Belgium.
E-mail addresses: koen.simons@vub.ac.be (K. Simons), tom.desmedt@wiv-isp.be (T. De Smedt), christophe.stove@ugent.be (C. Stove), peter.depaepe@ugent.be (P. De Paepe), michael.bader@basf.com (M. Bader), ben.nemery@med.kuleuven.be (B. Nemery), christiane.vleminckx@wiv-isp.be (C. Vleminckx), koen.decremer@wiv-isp.be (K. De Cremer), ilse.vanovermeire@wiv-isp.be (I. Van Overmeire), sebastien.fierens@wiv-isp.be (S. Fierens), birgit.mertens@wiv-isp.be (B. Mertens), thomas.goeen@ipasum.med.uni-erlangen.de (T. Göen), tschettgen@ukaachen.de (T. Schettgen), herman.vanoyen@wiv-isp.be (H. Van Oyen), joris.vanloco@wiv-isp.be (J. Van Loco), an.vannieuwenhuyse@wiv-isp.be (A. Van Nieuwenhuyse).
[1] Equal contribution.
[2] Scientific Institute of Public Health, J. Wytsmanstraat 14, 1050 Brussels, Belgium.
[3] Ghent University, Laboratory of Toxicology, Department of Bioanalysis, Ottergemsesteenweg 460, 9000 Ghent, Belgium.
[4] Ghent University Hospital, Department of Emergency Medicine, De Pintelaan 185, 9000 Ghent, Belgium.
[5] BASF SE, Occupational Medicine & Health Protection, GUA/CB-H308, 67056 Ludwigshafen, Germany.
[6] Katholieke Universiteit Leuven, Department of Public Health and Primary Care, Centre for Environment and Health, Herestraat 49, 3000 Leuven, Belgium.
[7] University of Erlangen-Nuremberg, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Schillerstrasse 25/29, 91054 Erlangen, Germany.
[8] RWTH Aachen University, Institute for Occupational and Social Medicine, Medical Faculty, Pauwelsstrasse 30, 52074 Aachen, Germany.

http://dx.doi.org/10.1016/j.envres.2016.03.031

smokers. Overall, the value of self-reported symptoms to assess exposure showed to be limited. The results of this study confirm that a critical view should be taken when considering self-reported health complaints and that ideally biomarkers are monitored to allow an objective assessment of exposure.

1. Introduction

In the night of Saturday May 4, 2013, a train transporting butadiene, triethylaluminium and acrylonitrile (ACN) derailed in the village of Wetteren (Belgium). Several rail tank cars with ACN exploded and a fire developed. Toxic fumes of ACN as well as hydrogen cyanide and nitrogen oxides were released due to the fire-induced decomposition of ACN (Van Nieuwenhuyse et al., 2014; De Smedt et al., 2014). To avoid explosion of the rail tank cars with butadiene and triethylaluminium, water was used to extinguish the fire and to cool the intact rail tanks. This water partly passed into the stream along the railway track and ended up in the sewers which resulted in a further distribution of ACN. This atypical sequence of events resulted in the evacuation of more than 2000 residents living in the close vicinity of the accident and along the sewage system. The duration of the evacuation period varied from 3 days up to 3 weeks, mainly depending on the distance from the residence to the accident site. One resident living next to the sewage system died and two other residents experienced life-threatening symptoms. In total, around two hundred inhabitants of Wetteren presented at the emergency services of the surrounding hospitals between May 4 and 14.

Both the physicochemical and toxicological properties of ACN are responsible for the impact of the train derailment. ACN (C_3H_3N) is a monomer used as an intermediate in the manufacturing of acrylic fibres, styrene plastics, and adhesives. At room temperature, ACN is a volatile, flammable, water-soluble, colourless liquid with a garlic or onion-like odour (EU Risk Assessment Report, 2004). ACN vapours are heavier than air and may thus travel along the ground over a long distance. Absorption of ACN may occur by inhalation, dermal contact, or oral ingestion and is rapid and extensive (Kedderis et al., 1993; Pilon et al., 1988; van Hooidonk et al., 1986). Following absorption, ACN is readily distributed throughout the body (Sandberg and Slanina, 1980). There is no evidence for significant accumulation of the substance itself in any organ. However, ACN can react at electrophilic sites of endogenous macromolecules, e.g. haemoglobin (Hb) proteins, and thus can generate adducts. Hb adducts accumulate during the life span of the red blood cells and thus can reflect the exposure during the past 4 months. Metabolism of ACN primarily takes place by two pathways (Gargas et al., 1995; Burka et al., 1994; Kedderis et al., 1993; Fennell et al., 1991; Dahl and Waruszewski, 1989), i.e. (i) conjugation with glutathione and (ii) oxidation by the cytochrome P450 isoenzyme CYP2E1. The first pathway results in the formation of an ACN-glutathione conjugate which will be further converted into a mercapturic acid, representing the final urinary excretion product. Consequently, this pathway is generally considered to be a detoxification step. Within the second pathway, the epoxide 2 cyanoethylene oxide (CEO) is formed as a primary metabolite. CEO is mutagenic and therefore this metabolic pathway is considered as the activation step. CEO can also undergo extensive secondary metabolism which includes the interaction with glutathione forming a series of cysteine or N-acetyl cysteine derivatives and the production of the highly toxic metabolite cyanide through the action of epoxide hydrolase. Cyanide can be detoxified by the mitochondrial enzymes rhodanese and mercaptopyruvate S-transferase to thiocyanate and excreted in the urine. The principal route of elimination for ACN, administered by oral or other routes, and its metabolites is urine with smaller amounts excreted in either the faeces or exhaled breath (Kedderis et al., 1993; Tardif et al., 1987; Ahmed et al., 1983). Acute toxicity of ACN mainly includes respiratory and neurological symptoms. ACN is an acute respiratory tract irritant causing effects such as irritation of the mucous membranes of the nose, eyes and upper respiratory tract. More serious exposures may lead to respiratory arrest and even death. Neurological symptoms may include limb weakness, dizziness, nausea and vomiting, headache, tremor, convulsions, coma and eventually death. The mode of action for neurological effects may involve both the parent chemical and the release of cyanide during metabolism. The mode of action for irritation effects is not known but may involve the binding of ACN or its primary metabolite to cellular macromolecules or depletion of tissue glutathione levels (ATSDR, 1990; WHO, 2002; AN Group, 2004).

Biomonitoring has been revealed as a powerful tool for the individual exposure assessment and risk estimation for citizens and rescue workers affected by chemical incidents (Müller et al., 2014, Scheepers et al., 2014). Particularly, Hb adducts excel for this task because of their long half-live, which enables exposure estimation also from samples withdrawn several days or weeks after the exposure scenario (Bader et al., 2014; Kloth et al., 2014; Leng and Gries, 2014).

We previously reported on the ACN exposure of the residents and emergency responders in a biomonitoring study in which *N*-2-cyanoethylvaline (CEV) in the venous blood was monitored (De Smedt et al., 2014; Van Nieuwenhuyse et al., 2014). CEV is the adduct formed by reaction of ACN with the N-terminal valine in human globin. This adduct is highly specific for exposure to ACN and, because it is built in erythrocytes, follows zero order kinetics after a single exposure event, gradually disappearing as the erythrocyte pool is being replaced, i.e. after 126 days in humans (Granath et al., 1992, Bader et al., 2014). Based on the CEV concentrations measured in blood, values were extrapolated by back-calculation to the concentrations that were to be expected at the time of the accident, i.e. May 4, using the formula: extrapolated CEV = measured CEV/$(1 - t \times 0.008)$, where "t" is the number of days between the accident and the blood sampling (Granath et al., 1992; Bader and Wrbitzky, 2006). As smoking is a known confounder for ACN exposure, cotinine measurements in urine were used to differentiate (Benowitz, 1996) between smokers (urinary cotinine > 100 μg/L) and non-smokers (urinary cotinine < 25 μg/L). For those in between, the smoking status was determined based on the self-reported questionnaire: 'smokers' and 'occasional smokers' were categorised as 'smokers', and 'ex-smokers' and 'non-smokers' as 'non-smokers'. Within these manuscripts, health effects of ACN exposure were not considered.

The objectives of the present study are therefore (1) to describe the short-term health effects that were reported by the evacuated residents following the train accident, and (2) to explore the association between the extrapolated CEV concentrations and the self-reported short-term health effects.

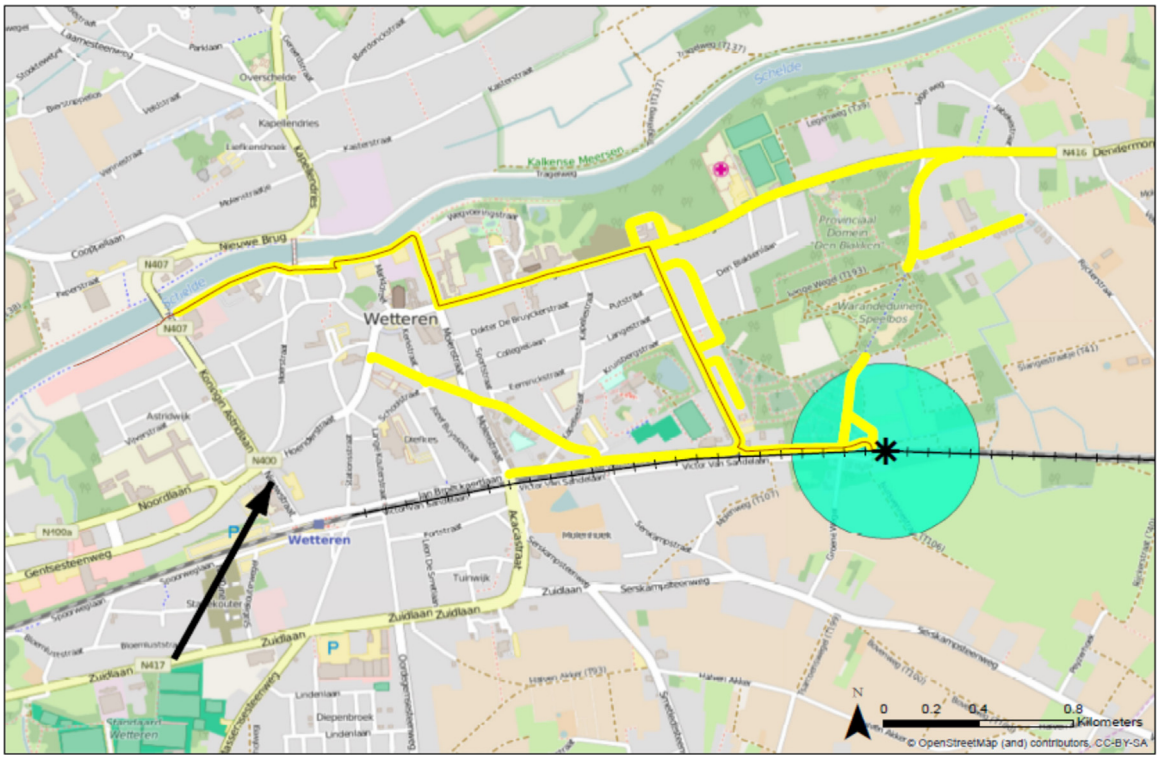

Fig. 1. Study area for the biomonitoring study in the local population. Legend of Fig. 1: Train accident. Railroad. Sewage system. Prevailing wind directions at the moment of and in the days following the accident. Zone 1 (EZ1): 250 m perimeter of the evacuation zone that was evacuated at night in the hours following the accident. Zone 2 (EZ2): streets parallel with the sewage system and downwind of the train accident that were evacuated later, i.e. in the days following the accident.

2. Materials and methods

2.1. Study population and methods

The evacuation zone (EZ) was defined by the Crisis Management Cell. The different zones are depicted in Fig. 1. Zone 1 corresponds to the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident. Zone 2 was evacuated later, i.e. in the three days following the accident, and included the streets parallel to the sewage system and the streets downwind of the train accident. Three groups of adult inhabitants of the EZ were invited to participate in the biomonitoring study. A first group consisted of residents of zone 1 (group 'EZ1'). A second group consisted of residents of zone 2 that were known to have presented at the emergency services of the surrounding hospitals (group 'EZ2 Emerg'). A third group consisted of a sample of the residents of zone 2 that had been evacuated, but had not visited the emergency services (group 'EZ2 Evac'). A sample of 10% of the households was taken and, within each household, one resident was invited to participate in the biomonitoring program, i.e. the person who was the first to have his birthday following the accident. In case the selected person was unable to attend the sampling, another member of the household was offered to participate in the biomonitoring program.

The biomonitoring study was carried out between May 18 and 25 2013 (days 14 till 21 after the accident). CEV adducts in venous blood were measured by a modified Edman degradation (Van Sittert et al., 1997; Tornqvist et al., 1986) and cotinine in the urine by online-SPE-UPLC®-MS/MS (De Cremer et al., 2013). The study protocol was approved by the Ethical Committee of Ghent University Hospital. An informed consent was signed by all participants prior to their participation in the study and the study was completed in accordance with the Helsinki declaration. In the evacuated zones, 197 residents participated in the biomonitoring study (participation rate of 55%). Out of these 197 residents, 191 also filled in the questionnaire with health symptoms. These 191 residents constitute the study population of the current study. The participation rates in EZ1 and in EZ2 Emerg were 65.0% and 47.5%, respectively. Of the eligible persons in EZ2 (one resident in a 10% sample of the households), 53.9% participated. Table 1 presents the descriptive statistics of the study population. Fifty-five persons were smokers and 136 were non-smokers. The median age in non-smokers was 49 years and 51 (37.5%) were men. In non-smokers, median CEV concentrations were similar between the three groups of residents. However, the variation was different, with a larger proportion of residents with high CEV concentrations in the groups that were evacuated later. In the group 'EZ2 Emerg', the 95th percentile and maximum were 2761 and 12,615 pmol/g globin, respectively. In the group 'EZ2 Evac', the 95th percentile and the maximum were 340 and 2129 pmol/g globin. In smokers, the median age was 40 years and 29 (52.7%) were men. No differences in CEV concentrations among the subgroups were observed in smokers.

2.2. Self-reported short-term health effects

Short-term health effects were assessed by means of a questionnaire distributed at the time of blood and urine collection, i.e. days 14–21 after the accident. A predefined list of health effects that have been described in relation to ACN exposure in literature (Vleminckx et al., 2014) was given to the participants. They were asked to fill in to what degree they had experienced each health effect following the accident: 'no symptoms', 'moderate symptoms', and 'serious symptoms'. The following health effects were

Table 1
Characteristics of the local study population in the evacuated zone of the train accident (n=191).

Non-smokers				
	EZ1 (n=20)	**EZ2 Emerg (n=33)**	**EZ2 Evac (n=83)**	**Total (n=136)**
Age (years), Median (IQR)	58.00 (39.75–69.25)	49.50 (35.00–58.00)	48.00 (34.00–62.50)	49.00 (35.00–64.00)
Men n (%)	8 (40%)	12 (36.4%)	31 (37.3%)	51 (37.5)
Extrapolated CEV (pmol/g globin)				
Median (IQR)	9.9 (4.0–14.4)	8.0 (5.7–67.6)	6.8 (3.4–14.7)	6.9 (3.4–16.2)
95th percentile	35.9	2760.6	74.7	623.3
Maximum	64.8	12,614.9	1461.4	12,614.9
> ref value n (%)	10 (50.0)	13 (39.4)	27 (32.5)	50 (36.8)
Smokers				
	EZ1 (n=6)	**EZ2 Emerg (n=14)**	**EZ2 Evac (n=35)**	**Total (n=55)**
Age (years), Median (IQR)	45.50 (35.50–48.75)	37.00 (33.75–48.75)	40.00 (31.00–54.00)	40.00 (32.00–53.50)
Men n (%)	4 (66.7%)	7 (50.0%)	18 (51.4%)	29 (52.7%)
Extrapolated CEV (pmol/g globin)				
Median (IQR)	230.3 (/)	185.8 (105.3–287.5)	174.3 (141.6–264.6)	183.0 (149.5–272.5)
95th percentile	/	355.6	477.3	402.4
Maximum	337.5	373.9	694.8	694.8
> ref value n (%)	2 (33.3)	6 (42.9)	14 (40.0)	22 (40.0)

EZ1 – Residents of zone 1 of the EZ, i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident.
EZ2 – Residents of zone 2 of the EZ, i.e. the streets parallel with the sewage system and downwind of the train accident that was evacuated in the days following the accident.
EZ2 Emerg – Residents of EZ2 that were known to have presented at the emergency services.
EZ2 Evac – Residents of EZ2 that were evacuated, but did not visit the emergency services. A sample of 10% of the households was taken and, within each household, one resident was invited to participate in the biomonitoring program, i.e. the person who was the first to have his birthday following the accident. In case the selected person was unable to attend the sampling, another member of the household was offered to participate in the biomonitoring program.
IQR=interquartile range.
CEV – *N*-2-cyanoethylvaline.

included in the list: coughing, eye irritation, skin irritation, irritation of the nose, throat or airways, headache, nausea, and tremor. Participants could also indicate if they had experienced 'other' health effects than the ones mentioned and, in that case, describe their symptoms.

For the analyses, four categories of symptoms were created: (i) irritation (Category 1) including coughing, eye irritation, skin irritation, and irritation of the nose, throat or airways; (ii) headache (Cat 2); (iii) nausea (Cat 3); and (iv) tremor (Cat 4). Prevalence of these symptom categories was calculated, stratified by smoking status ('smoker' – 'non-smoker') and subgroup ('EZ1', 'EZ2 Emerg', 'EZ2 Evac'). Because the relationship between ACN exposure and symptoms in humans has not been studied before and there is a lack of data to rely on, a non-parametric approach was chosen to explore the association between CEV concentrations and self-reported symptoms. Analyses were carried out separately for non-smokers and smokers. 'Moderate' and 'serious' symptoms were collapsed into one category to increase statistical power. In a first step, the independence between the CEV concentrations and all symptom categories together was tested, using an omnibus test of independence based on the sample distance covariance (Szekely et al., 2007). In a second step, the same test was applied to verify independence between ACN exposure and each category of symptoms separately. In a last step, the dose-response relationship between the CEV concentrations and the short-term health effects was quantified for each symptom that was significant in Step 2. To this purpose, Generalized Additive Models (GAMs) were used. GAMs (Hastie and Tibshirani, 1990) are regression models that do not make assumptions on the format of the dose-response relationship itself (non-parametric part), but they do allow to account for different baseline risks in function of the subgroups (parametric part). This was necessary because the baseline risk for reporting symptoms showed to be different by subgroup (see further). As such, for each significant symptom of Step 2, a GAM model was fitted with the following linear predictor:

$$logit(\pi) = f(exposure) + group_a + group_b + group_c$$
$$y \sim Binomial(\pi),$$

where the logit link function is used with the symptom risk π as the outcome y is binary, f is a smooth function of the exposure using thin-plate splines, and there are three dummy variables for the three subgroups.

3. Results

3.1. Self-reported symptoms following the train accident (Table 2)

The most prevalent symptoms in the 191 evacuated participants were irritation (48.5% in non-smokers and 65.5% in smokers) and headache (39.0% in non-smokers and 56.4% in smokers). Nausea and tremor were reported by 16.2% and 5.1% of the non-smokers, respectively. In smokers, the prevalence of nausea and tremor were both 12.7%. Both in non-smokers and smokers, nose, throat and airways problems were the most prevalent irritation complaints, followed by eye irritation and coughing. Skin irritation was reported by 5% or less of the participants. In non-smokers, health symptoms were reported more frequently by the residents who had presented at the emergency services (EZ2 Emerg), whereas a mixed pattern was seen in smokers.

3.2. Association between CEV concentrations and short-term health effects

3.2.1. Testing for independence (Table 3)

In the group of smokers, no association was seen between the CEV concentrations and the four symptom categories together (p=0.400). In non-smokers, however, dose-dependency was observed between ACN exposure at the time of the train accident and the self-reporting of short-term health effects (p=0.007).

When testing for each symptom category separately in non-smokers, the distance-covariance test gave significant results for

Table 2
Self-reported symptoms of the study population in the evacuated zone of the train accident (n=191).

Non-smokers				
	EZ1 (n=20) n (%)	**EZ2 Emerg (n=33) n (%)**	**EZ2 Evac (n=83) n (%)**	**Total (n=136) n (%)**
Irritation (Cat1)	9 (45.0)	25 (75.8)	32 (38.6)	66 (48.5)
Headache (Cat2)	5 (25.0)	19 (57.6)	29 (34.9)	53 (39.0)
Nausea (Cat3)	3 (15.0)	14 (42.4)	5 (6.0)	22 (16.2)
Tremor (Cat4)	2 (10.0)	2 (6.1)	3 (3.6)	7 (5.1)
Reported complaints of irritation (Cat1)				
Coughing	6 (30.0)	9 (27.3)	10 (12.0)	25 (18.4)
Eye irritation	2 (10.0)	8 (24.2)	16 (19.3)	26 (19.1)
Skin irritation	0(0.0)	3 (9.1)	2 (2.4)	5 (3.7)
Irritation of the nose, throat, and airways	5 (25.0)	17 (51.5)	21 (25.3)	53 (31.6)
Smokers				
	EZ1 (n=6) n (%)	**EZ2 Emerg (n=14) n (%)**	**EZ2 Evac (n=35) n (%)**	**Total (n=55) n (%)**
Irritation (Cat1)	5 (83.3)	12 (85.7)	19 (54.3)	36 (65.5)
Headache (Cat2)	5 (83.3)	8 (57.1)	18 (51.4)	31 (56.4)
Nausea (Cat3)	1 (16.7)	3 (21.4)	3 (8.6)	7 (12.7)
Tremor (Cat4)	1 (16.7)	3 (21.4)	3 (8.6)	7 (12.7)
Reported complaints of irritation (Cat1)				
Coughing	4 (66.7)	6 (42.9)	7 (20.0)	17 (30.9)
Eye irritation	3 (50.0)	5 (35.7)	10 (28.6)	18 (32.7)
Skin irritation	0 (0.0)	0 (0.0)	3 (8.6)	3 (5.5)
Irritation of the nose, throat, and airways	3 (50.0)	10 (71.4)	16 (45.7)	29 (52.7)

Table 3
Association between CEV concentrations and self-reported symptoms in the evacuated residents.

	Null hypothesis of independency[a] (P value)	
	Non-smokers (n=136)	Smokers (n=55)
Irritation or headache or nausea or tremor	**0.007**	0.400
Irritation (Cat 1)	**0.007**	/
Headache (Cat 2)	0.882	/
Nausea (Cat 3)	**0.007**	/
Tremor (Cat 4)	0.081	/

[a] Omnibus test based on the sample distance covariance.

irritation (Cat1, p=0.007) and nausea (Cat3, p=0.007). Results were non-significant for headache (Cat2, p=0.882) and tremor (Cat4, p=0.081); hence, these symptom categories were not explored further.

3.2.2. *Quantification of the dose-response relationship*

For each subgroup of non-smokers, the dose-response relationship was estimated between the CEV concentrations at the time of the accident and the reporting of irritation (Cat1) and nausea (Cat3). Fig. 2a and b show the results for irritation (Cat1) and nausea (Cat3), respectively. For information, the scatterplots are added in Fig. 3. From Fig. 2, the following results were found:

1) The dose-response relationship between the CEV concentrations in the blood of the residents at the time of the accident and the reporting of irritation and nausea was best fitted by a monotonously increasing relation.
2) The probability to report symptoms by residents with CEV concentrations below the reference value of 10 pmol/g globin (Kraus et al., 2016), further called 'the baseline risk', was not zero. The baseline risk was higher for irritation than for nausea. This observation already became clear from the scatterplots (Fig. 1): irritation and nausea were also reported by residents with CEV concentrations below the reference value of 10 pmol/g globin, although reporting was more pronounced for irritation. This lack of pattern was clear up to CEV concentrations of 100 pmol/g globin.
3) For residents with high CEV concentrations, i.e. 100 pmol/g globin or higher, the probability to report symptoms was very high for irritation, but remained lower for nausea. This pattern can also be seen in the scatterplots (Fig. 3): almost all residents with CEV concentrations above 100 pmol/g globin reported irritation symptoms. Nausea was less reported, even when residents had high CEV concentrations.
4) The probability to report symptoms differed clearly among the subgroups; it was highest in the group EZ2 Emerg. In the groups EZ2 Evac and EZ1, the probability to report symptoms was similar, but lower as compared to EZ2 Emerg. The broadest range of CEV concentrations was also observed in the EZ2 Emerg group (ranging from 1.3 up to 12,610.0 pmol/g globin) as compared to EZ2 Evac (1.3 up to 1461.0 pmol/g globin) and EZ1 (1.3 up to 64.8 pmol/g globin). More symptoms were reported in the EZ2 Emerg group. This trend was observed for the whole range of CEV concentrations, and thus independently of the dose.

4. Discussion and conclusions

The most prevalent complaints in the evacuated residents were local symptoms of irritation. This is in line with literature, irritation being an important effect of ACN (EU Risk assessment Report, 2004). Almost all non-smokers with CEV concentrations above 100 pmol/g globin reported irritation symptoms. Irritation can thus be considered as a rather sensitive symptom for more pronounced ACN exposures in non-smokers. However, irritation was also substantially reported by non-smokers with CEV concentrations below the reference value of 10 pmol CEV/g globin. This was somewhat unexpected, as non-smokers with CEV concentrations below the reference value are considered to fall within the 95th percentile of a non-exposed population. The symptom reporting in non-exposed people renders irritation a non-specific symptom for lower ACN exposures. The inconsistent reporting of symptoms was observed up to CEV concentrations of 10 times the reference value. Several hypotheses may be put forward to explain the reporting of symptoms in non-exposed people and people exposed to low concentrations of ACN. First, some residents may have experienced very short peak exposures to ACN that are not reflected by the adduct biomonitoring due to its low temporal resolutions. Second, other vapours (not ACN) and dusts in connection with the accident might have provoked these symptoms. In non-smokers with CEV concentrations below 100 pmol/g globin who reported irritation, the most reported complaints were coughing (43.6%) and irritation of the nose, throat and airways (70.9%) (additional analyses, results not shown). Upper airway irritation may point very well to the

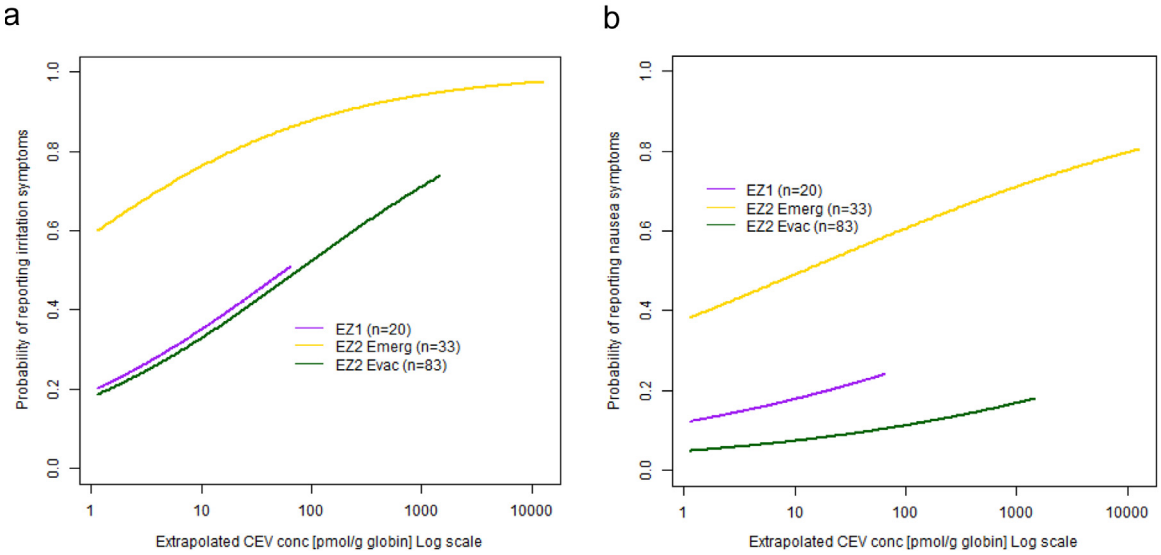

Fig. 2. Dose-response relationship between the CEV concentrations at the time of the accident and short-term health effects.

inhalation of exhausts and vapours. In the specific context of this train accident, it needs to be remarked that few or no people will have experienced exposure to smoke from the derailment, given the distance to the accident site and the wind direction, blowing away from the evacuated zone (Fig. 1). Hence, there is no basis to conclude that an increased exposure to e.g. dust or smoke took place. Third, the reporting of symptoms by residents with lower CEV concentrations may be suggestive for anxiety and mass sociogenic illness, or induced by communication. Indeed, in the media, in which the derailment received extensive coverage during multiple days, irritation was communicated as the most important symptom that may be experienced following exposure to ACN.

Among the systemic symptoms, headache was reported in the same order of magnitude as the local symptoms, i.e. by 40–60% of the residents. Nausea and tremor were reported by less than 20% of the residents. In non-smokers, a (monotonous) dose-response relationship was seen between the ACN exposure at the time of the train accident and the reporting of nausea. The results for nausea, however, were less conclusive than for irritation: the probability to report nausea by non-smokers with CEV concentrations above 100 pmol/g globin was lower than for irritation. Furthermore, both absence and presence of symptoms was reported by non-smokers with CEV concentrations below the reference value and up to 10 times the reference value. Therefore, nausea seems to be a less sensitive symptom than irritation in case of higher ACN exposure and a non-specific symptom in non- or less exposed people. For headache and tremor, no dose-response relationships were observed between ACN exposure at the time of the train accident and symptom reporting. All three systemic symptoms may be due to other reasons such as increased stress and agitation, and are therefore not exclusively related to exposure to chemical substances.

Out of the 3 non-smoking subpopulations of the EZ, the probability to report symptoms was clearly higher in the residents that presented at the emergency services (EZ2 Emerg). In this group, the highest CEV concentrations were also observed. The pattern of increased symptom reporting in the EZ2 Emerg, however, was seen for the whole range of CEV concentrations, and is thus independently of the dose. This may point to residents who are more vulnerable to the effects of ACN, e.g. due to underlying conditions, or to residents who go more easily to the emergency department, independently of their medical condition. As such,

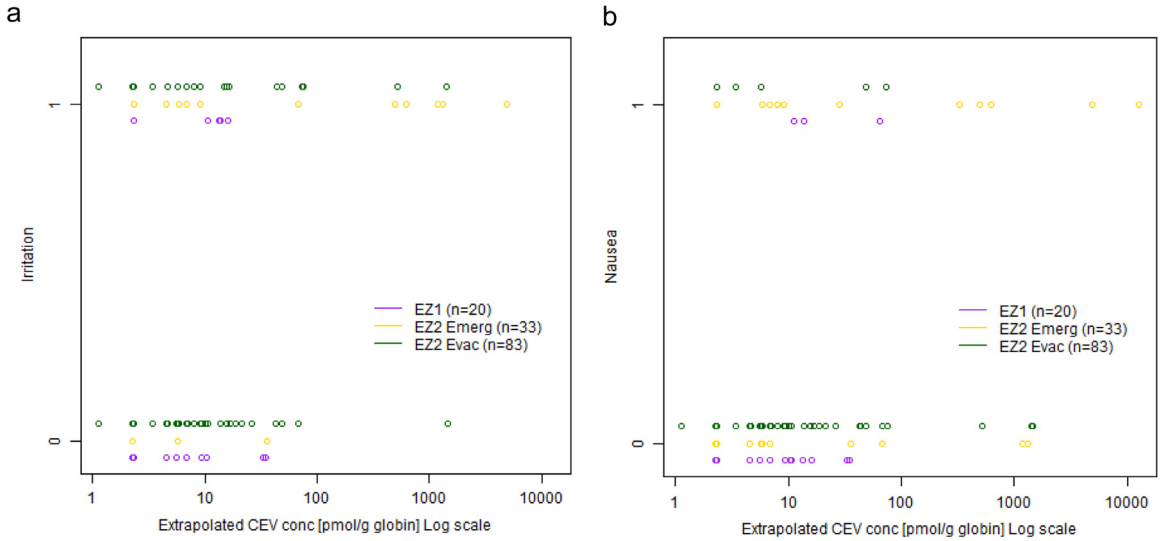

Fig. 3. Scatterplots of CEV concentrations by self-reported symptoms.

limiting the study population for intoxications to the persons presenting at the emergency services is not a good practice from public health point of view. Although the residents with severe symptoms were clearly captured, it is unknown how many persons with increased concentrations did not present themselves to the hospital. This number may be important. On the other hand, the number of persons that showed up in the hospitals without clear exposure-associated symptoms is unclear (Gunnell, 1993). A similar phenomenon was observed in persons contacting an emergency helpline that was installed by the health authorities following a chemical contamination of the water supply. Fone and co-workers concluded that the helpline was primarily used by people who were 'worried well', regardless of the incident or any exposure that had occurred (Fone et al., 1998).

Symptoms in the present study, as with any other study like this, were not scored by a medical doctor, but were self-reported by the person involved. Furthermore, the questionnaire was distributed at the moment of the blood and urine sampling, thus between days 14 and 21 after the accident. This may have led to some information bias.

A significant association between CEV concentrations at the time of the accident and short-term health effects was found in non-smokers, but not in smokers. This lack of association in smokers may be caused by the higher background CEV concentrations in this population. Furthermore, smokers are more exposed to vapours of chemicals triggering irritation and therefore, a further increase in irritation due to ACN exposure may not be perceived by this population group.

Scientific studies in the acute context of public health emergencies are limited. When a crisis occurs, the first priority is the management of the crisis, science lagging far behind. Some studies have reported on the occurrence of self-reported health symptoms following chemical incidents (Sim et al., 2010; Na et al., 2013; Tjalvin et al., 2015; Wilken et al., 2015), or on the relationship between health surveys and chemical exposures as measured in environmental matrices (Fowle et al., 1996; Arnedo-Pena et al., 2003). In general, the validity of self-reported symptoms in exposure assessment is subject to information bias by phenomena as anxiety and mass socio-genic illness (Gallay et al., 2002). Even in well-designed case-control studies, it is difficult to interpret whether an increased exposure reported by the cases as compared to the controls, points to a true increase of exposure or is due to bias. Selection of cases and controls in such studies is usually done based on self-reported symptoms, cases being persons who report symptoms and controls being persons who report no or less symptoms. Consequently, there is always an uncertainty due to the fact that persons considered as 'cases' are just self-reporting more symptoms and exposure than persons included in the control group (Nemery et al., 2002). In our study, we had the opportunity to investigate the relationships between self-reported symptoms and individual exposure to chemical contaminants as directly measured by biomarkers. Our study confirms the limited value of self-reported symptoms to assess exposure, with exception of some local symptoms that are known to be prominent for the specific chemical compound studied. Even then, the reporting of symptoms was only absolute in case of exposures that resulted in CEV values exceeding 10 times the reference value. For the lower exposure ranges, there was no clear relationship between symptom reporting and exposure. From a public health point of view, however, it is relevant to know whether persons have been exposed, even at low or moderate concentrations (Hahn et al., 2012).

In conclusion, the present study is one of the first to relate exposure levels to a chemical released during a chemical incident to short-term (self-reported) health effects. A thorough analysis of exposure and effects allowed for an adequate scientific evaluation of the health impact in connection with the accident, and provided a basis for a fact-based public communication. The results of this study confirm that a critical view should be taken when considering self-reported health complaints and effects in the aftermath of a chemical disaster and that ideally biomarkers are monitored to allow an objective assessment of exposure.

Acknowledgements

This study has been financed by the FPS Health, Food Chain Safety and Environment, following an advice of the Belgian Minister of Social Affairs and Public Health. The funding source was responsible for the logistical organisation of the study. The authors thank the inhabitants of Wetteren for their participation in the study and the local practitioners for their assistance in the sampling and their close involvement throughout the whole study. The authors thank Geert Gijs, crisis coordinator of the FPS Health, Food Chain Safety and Environment, and his team for the logistical organisation of the study. The authors are grateful to Wesley Van Dessel and Jan Eyckmans, respective heads of the communication services of the WIV-ISP and of the FSP Health, Food Chain Safety and Environment, and their team members, for the continuous support in the communication of the study and its results. The authors also want to thank Stéphanie Fraselle and her colleagues (WIV-ISP) for the preparation of the blood samples before sending them to the German labs. Finally, the authors thank Sabine Janssens and Tadek Krzywania and his team (WIV-ISP) for the enormous efforts with regard to data input, data processing and administrative support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.03.031.

References

Ahmed, A.E., Farooqui, M.Y.H., Upreti, R.K., El-Shabrawy, O., 1983. Comparative toxicokinetics of 2, 3014C- and 1–14C-acrylonitrile in the rat. J. Appl. Toxicol. 3, 39–47.

AN Group, 2004. Toxicological Review of Acrylonitrile (CAS No. 107-13-1). Dayton, OH, Sapphire Group. Available at: ⟨www.tera.org/Peer/AN/ANToxicologicalReviewDocument-Revised.pdf⟩.

Arnedo-Pena, A., Bellido-Blasco, J., Villamarin-Vazquez, J.L., Aranda-Mares, J.L., Font-Cardona, N., Gobba, F., Kogevinas, M., 2003. Acute health effects after accidental exposure to styrene from drinking water in Spain. Environ. Health 2, 6.

ATSDR, 1990. Agency for Toxic Substances and Diseases Registry, US Public Health Service Toxicological Profile: Acrylonitrile. ⟨http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=447&tid=78⟩.

Bader, M., Wrbitzky, R., 2006. Follow-up biomonitoring after accidental exposure to acrylonitrile – implications for protein adducts as a dose monitor for short-term exposure. Toxicol. Lett. 162, 131.

Bader, M., Van Weyenbergh, T., Verwerft, E., Van Pul, J., Lang, S., Oberlinner, C., 2014. Human biomonitoring after chemical incidents and during short-term maintenance work as a tool for exposure analysis and assessment. Toxicol. Lett. 231, 328–336.

Benowitz, N.L., 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev. 18, 188–204.

Burka, L.T., Sanchez, I.M., Ahmed, E.A., Ghanayem, B.I., 1994. Comparative metabolism and deposition of acrylonitrile and methacrylonitrile in rats. Arch. Toxicol. 68, 611–618.

Dahl, A.R., Waruszewski, B.A., 1989. Metabolism of organonitriles and cyanide by rat nasal tissue enzymes. Xenobiotica 19, 1201–1205.

De Cremer, K., Van Overmeire, I., Van Loco, J., 2013. On-line solid-phase extraction with ultra performance liquid chromatography and tandem mass spectrometry for the detection of nicotine, cotinine and trans-3′-hydroxycotinine in urine to strengthen human biomonitoring and smoking cessation studies. J. Pharm. Biomed. Anal. 76, 126–133.

De Smedt, T., De Cremer, K., Vleminckx, C., Fierens, S., Mertens, B., Van Overmeire, I., Bader, M., De Paepe, P., Göen, T., Nemery, B., Schettgen, T., Stove, C., Van Oyen, H., Van Loco, J., Van Nieuwenhuyse, A., 2014. Acrylonitrile exposure in the

general population folowing a major train accident in Belgium: a human biomonitoring study. Toxicol. Lett. 231, 344–351.
EU Risk assessment Report, 2004. Acrylonitrile, Institute for Health and Consumer Protection, European Chemicals Bureau, EUR 20857 EN.
Fennell, T.R., Kedderis, G.L., Sumner, S.C.J., 1991. Urinary metabolites of [1,2,3-13C] acrylonitrile in rats and mice detected by 13C nuclear magnetic resonance spectroscopy. Chem. Res. Toxicol. 4, 678–687.
Fone, D., Constantine, C., McCloskey, B., 1998. The Worcester water incident, UK: bias in self reported symptoms to an emergency helpline. J. Epidemiol. Commun. Health 52, 526–527.
Fowle, S., Constantine, C., Fone, D., McCloskey, B., 1996. An epidemiological study after a water contamination incident near Worcester, England in April 1994. J. Epidemiol. Commun. Health 50, 18–23.
Gallay, A., Van Loock, F., Demarest, S., Van der Heyden, J., Jans, B., Van Oyen, H., 2002. Belgian Coca-Cola-related outbreak: intoxication, mass sociogenic illness, or both? Am. J. Epidemiol. 155, 140–147.
Gargas, M.L., Anderson, M.E., Teo, S.K.O., Batra, R., Fennell, T.R., Kedderis, G.L., 1995. A physiologically-based dosimetry description of acrylonitrile and cyanoethylene oxide in the rat. Toxicol. Appl. Pharm. 134, 185–194.
Granath, F., Ehrenberg, L., Tornqvist, M., 1992. Degree of alkylation of macromolecules in vivo from variable exposure. Mutat. Res. 284, 297–306.
Gunnell, D., 1993. The public health physician's role in chemical incidents. J. Public Health Med. 15, 352–357.
Hastie, T.J., Tibshirani, R.J., 1990. Generalized Additive Models. Chapman & Hall/CRC.
Hahn, A., Michalak, H., Begemann, K., Meyer, H., Burger, R., 2012. Expert judgement of poisonings and human biomonitoring – the BfR three-level and matrix model. Int. J. Hyg. Environ. Health 215, 242–246.
Kedderis, G.L., Batra, R., Koop, D.R., 1993. Epoxidation of acrylonitrile by rat and human cytochromes P450. Chem. Res. Toxicol. 6, 866–871.
Kloth, S., Baur, X., Göen, T., Budnik, L.T., 2014. Accidental exposure to gas emissions from transit goods treated for pest control. Environ. Health 13, 110.
Kraus, T., Angerer, J., Bader, M., Schettgen, T., 2016. Addendum to acrylonitrile. The MAK-Collection for Occupational Health and Safety, 1 pp.
Leng, G., Gries, W., 2014. Biomonitoring following a chemical incident with acrylonitrile and ethylene in 2008. Toxicol. Lett. 231, 360–364.
Müller, M., Schmiechen, K., Heselmann, D., Schmidt, L., Göen, T., 2014. Human biological monitoring – a versatile tool in the aftermath of a CBRN incident. Toxicol. Lett. 231, 306–314.
Na, Y.H., Woo, K.H., Yoon, S.Y., Cho, S.Y., Song, I.U., Kim, J.A., Kim, J.S., 2013. Acute symptoms after a community hydrogen fluoride spill. Ann. Occup. Environ. Med. 25, 17.
Nemery, B., Fischler, B., Boogaerts, M., Lison, D., Willems, J., 2002. The Coca-Cola incident in Belgium, June 1999. Food Chem. Toxicol. 40, 1657–1667.
Pilon, D., Roberts, A.E., Rickert, D.E., 1988. Effect of glutathione depletion on the uptake of acrylonitrile vapors and on its irreversible association with tissue macromolecules. Toxicol. Appl. Pharmacol. 95, 265–278.
Sandberg, E.C., Slanina, P., 1980. Distribution of (1-14C) acrylonitrile in rat and monkey. Toxicol. Lett. 6, 187–191.
Scheepers, P.T., van Brederode, N.E., Bos, P.M., Nijhuis, N.J., van de Weerdt, R.H., van der Woude, I., Eggens, M.L., 2014. Human biological monitoring for exposure assessment in response to an incident involving hazardous materials. Toxicol. Lett. 231, 295–305.
Sim, M.S., Jo, I.J., Song, H.G., 2010. Acute health problems related to the operation mounted to clean the Hebei Spirit oil spill in Taean, Korea. Mar. Pollut. Bull. 60, 51–57.
Szekely, G.J., Rizzo, M.L., Bakirov, N.K., 2007. Measuring and testing dependence by correlation of distances. Ann. Stat. 35, 2769–2794.
Tardif, R., Talbot, D., Grin, M., Brodeur, J., 1987. Urinary excretion of mercapturic acids and thiocyanate in rats exposed to acrylonitrile: Influence of dose and route of administration. Toxicol. Lett. 39, 255–261.
Tjalvin, G., Hollund, B.E., Lygre, S.H., Moen, B.E., Bråtveit, M., 2015. Subjective health complaints among workers in the aftermath of an oil tank explosion. Arch. Environ. Occup. Health 70, 332–340.
Tornqvist, M., Mowrer, J., Jensen, S., Ehrenberg, L., 1986. Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. Anal. Biochem. 154, 255–266.
Van Hooidonk, C., 1986. The permeation of 14C-acrylonitrile through guinea pig skin and human skin in vitro and the effect of dermal protection (gloves, barrier creams) on permeation. TNO Report PML, 1986 C 103, Rijswijk, The Netherlands (in Dutch).
Van Nieuwenhuyse, A., Fierens, S., De Smedt, T., De Cremer, K., Vleminckx, C., Mertens, B., Van Overmeire, I., Bader, M., De Paepe, P., Göen, T., Nemery, B., Schettgen, T., Stove, C., Van Oyen, H., Van Loco, J., 2014. Acrylonitrile exposure assessment in the emergency responders of a major train accident in Belgium: a human biomonitoring study. Toxicol. Lett. 231, 352–359.
Van Sittert, N., Angerer, J., Bader, M., Blaszkewicz, M., Ellrich, D., Krämer, A., Lewalter, J., 1997. 2-Cyanoethylvaline, N-2-Hydroxyethylvaline, N-Methylvaline (as evidence of exposure to acrylonitrile, ethylene oxide and methylating agents). Angerer, J. Weinheim, VCH Verlagsgesellschaft. Analyses of hazardous substances in biological materials. Deutsche Forschungsgemeinschaft.
Vleminckx, C., Fierens, S, Verschaeve, L., Van Overmeire, I., Voisin, C., Mertens, B., De Cremer, K., Blaude, M.N., Van Nieuwenhuyse, A., 2014. Acrylonitrile Literature Review. WIV-ISP Report, D/2014/2505/36.
Wilken, J.A., Graziano, L., Vaouli, E., Markiewicz, K., Helverson, R., Brinker, K., Shumate, A.M., Duncan, M.A., 2015. Exposures and symptoms among workers after an offsite train derailment and vinyl chloride release. Am. J. Disaster Med. 10, 153–165.
WHO, 2002. Acrylonitrile: Concise International Chemical Assessment Documents (CICAD) 39. Available at: ⟨www.who.int/ipcs/publications/cicad/cicad39_rev.pdf⟩.